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Protozoa From New Zealand Termites.

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INTRODUCTION.

The intestinal fauna of the termites of New Zealand has not up to date been dealt with fully. Helson (1935) described a new protozoon belonging to the order Hypermastigina, *Spirotrichosoma magna* from *Stolotermes ruficeps* Brauer. There are two termites endemic to New Zealand, *Stolotermes ruficeps* and *Calotermes brouni.* Helson did not state a locality for his material, but there is reason to believe that it was obtained from Westland. Material for this paper was collected from Banks Peninsula, where both *S. ruficeps* and *C. brouni* were present in *Podocarpus totara* and *P. spicata* and one colony of *S. ruficeps* was recorded from *Griselinia littoralis;* also both genera were collected in the Nelson district; *C. brouni* from *Laurelia novae zelandia* and from the weather boards of an old farmhouse where it had caused considerable damage, and *S. ruficeps* from *Metrosideros lucida.* Material of each genus was often collected from adjacent logs and indeed, in one case, both from one log where the sampled areas were 20in apart. In every case each genus had its characteristic fauna and no intermingling of the protozoa was found, in fact an attempt to cause the fauna of the two genera to intermingle was unsuccessful in the laboratory. *S. ruficeps* was also obtained from the Teremakau Valley, Westland.

The flagellates described are Polymastigotes belonging to the families Devescovenidae, Oxymonadidae, Joenididae and Trichomonadidae.

MATERIAL AND TECHNIQUE.

Studies on living material were made by diluting the contents of the gut in either 0·75% saline or Ringer's solution. The large flagellates kept their shape for a much longer period in saline, which was most useful for a general study. On the other hand, in Ringer's solution the protoplasm tended to disintegrate after half an hour, leaving the neuromotor system intact, which was then more easily studied. The three small flagellates behaved equally well in both solutions.

Fixatives used were: Schaudinn's without acetic, Bouin's, Flemming's without acetic, Zenker's and Osmic vapour. Mallory's triple, Heidenhain's iron-alum haemotoxylin, Delafield's haemotoxylin and Erhlich's triple stains were used. Eosin or acid fuchsin was used as counter-stain for Heidenhain's iron-alum. Neutral red and methylene blue were used as intra vitam stains while alcoholic iodine served for emphasising flagella.

The best results in general observation were obtained with living material. A trace of alcoholic iodine in the saline or Ringer's solution rendered the flagellate clear and transparent so that the internal structures were clearly visible.

The best preparations were obtained from material fixed in Schaudinn's or Zenker's and followed by Mallory's triple stain, and Osmic vapour followed by Heidenhain's iron-alum haemotoxylin counter-stained with acid-fuchsin or eosin. Erhlich's Triple stain picked out particularly wood particles and the nucleus.

Caduceia calotermidis n.sp.

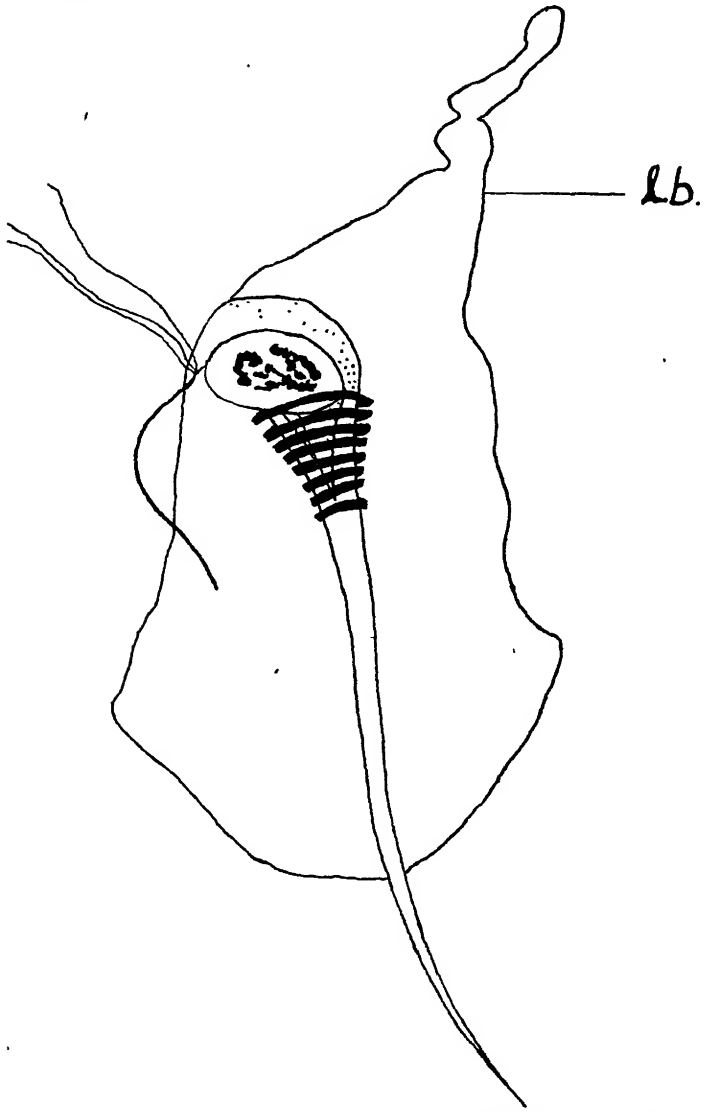
Host: Calotermes brouni.

Diagnosis: Length, 56μ–84μ; width, 33·6μ–47μ; three anterior flagella; trailing flagellum slender cord, less than body length; no cresta visible; parabasal body single spiral 5–11 coils (mostly 7 or 8 coils), fairly tight; capitulum of axostyle with granules (chromatic shield); nucleus 10·7μ x 7·6μ in large forms; rod-shaped micro-organisms adherent to parts of surface; spirochaetes 4μ–6μ long forming a dense coat over most of surface.

In the unstained living condition the flagellate is clear, the structures and wood particles within the cytoplasm being quite visible. The shape of the body is generally ovoid or pyriform, but it is also capable of changing. There is an axostyle present which projects posteriorly, sometimes to the length of 33·6μ. The size varies from 56μ–84μ long and 33·6μ–47·6μ wide. The anterior end is rounded on one side and prolonged into a snout on the other, from which three flagella of equal length project (Pl. 42, Figs. 2 and 3). There is a fourth trailing flagellum, a little stouter than the other three, which arises from the same place and projects backwards. It is about half the length of the body. The anterior portion, which has the appearance of a "head" with a projecting snout, is highly mobile, and at the same time the anterior flagella are lashed about in unison towards and away from the body. The fourth, trailing flagellum is vibrated actively and continuously. Nearly the whole of the organism is invested with spirillum-like bacteria 4μ–6μ in length (Pl. 42, Fig. 1) like those which have been called spirochaetes by Dobell (1910) and Damon (1926). Janicki, quoted by Kirby in his description of *Devescovina striata* var. *hawiensis,* mentions the spirillum-like bacteria investing the surface. Similarly, Kirby on *D. lemniscata* (1926) and Light (1926) on *Metadevescovina debilis* mention the bacteria adhering to the surface.

These spirochaetes were not removed by a weak tincture of iodine in 70% alcohol (Light, 1926), but were only removed after feeding on filter paper soaked in ·5% acid Fuchsin for a month (Cleveland, 1928). After this treatment the surface of the protozoa was quite free from spirillum-like organisms but there were also rod-like structures arranged in groups on the surface (Pl. 42, Fig. 1), which gave the appearance of striations. These were seen to swim off, then attach themselves again, and were obviously separate organisms. When invested with spirochaetes the flagellates moved along smoothly and vigorously, sometimes revolving on the longitudinal axis. The spirochaetes were directed posteriorly and appeared to be beating uniformly. When divested of its spirochaetes, the organism barely moved. The movement which took place seemed to be due to changes in shape of the body and the movement of the mobile head region with the flagella. Some of the flagellates were seen to send out extremely active lobopodia from the anterior end

(Text Fig. 1, lb.) and at the same time the cytoplasm underwent rapid shimmering movement. With the movement of the mobile "head" region the parabasal body and axostyle were turned also.



TEXT FIG. 1.—Living specimen of *Caduceia calotermidis* n.sp. from which the spirochaetes have been removed by acid fuchsin.

The movement of *C. calotermidis* when covered with spirochaetes corresponds exactly with Kirby's description of *Pseudodevescovina uniflagellata* (1936, pp. 313–314). He draws attention to the vigorous movement of the animal, which far surpasses *Stephanonympha*, an animal completely covered with flagella, and yet *P. uniflagellata* possesses such an apparently feeble locomotor apparatus. It is similar

to *C. calotermidis,* possessing the anterior flagella and a slightly stouter trailing flagellum which, he states, could hardly be responsible for the rapid motion. In the light of the feeble locomotion of *C. calotermidis* when naked as compared with the rapid and vigorous movement when covered with spirochaetes, it is suggested that they play a part in locomotion.

The axostyle, which is clearly visible, is a hyaline flexible rod which runs from the anterior end, just behind the mobile portion, to the posterior end from which it projects variously. The anterior portion of the axostyle is tri-radiate and situated part way in this inverted tetrahedron is the nucleus (Pl. 42, Fig.2). The nucleus is scarcely visible in life, but can be seen as a greyish ovoid body. The axostyle appears to continue anteriorly over one side of the nucleus and goes to form part of the head region. This continuation of the axostyle is called the capitulum (Pl. 42, Figs. 2 and 3 cp.) by Kirby (1936). This region is granular, and the granulation continues over one side of the nucleus into the region lying between the three rays of the axostyle (Pl. 42, Figs. 2, 3 and 4). From the base of the tri-radiate portion, the axostyle (though nearly cylindrical in this region) tapers to a point posteriorly.

Wrapped around the anterior portion of the axostyle is the clearly visible parabasal body in the form of a spiral in a counter-clockwise direction which is wide anteriorly and tapers posteriorly (Pl. 42, Figs. 2, 3 and 4). The band of the spiral is wide, being ·7μ, and there are up to 11 turns in some specimens, most having 7 or 8. Anteriorly the spiral continues as a fine thread to the point from which the four flagella arise (Pl. 42, Figs. 2 and 3).

The cytoplasm round about the axostyle, from the spiral backwards is dense and contains wood particles and food vacuoles (Pl. 42, Fig. 2). One food vacuole was observed to have living bacteria and spirochaetes in it. There is a fairly wide margin of clear ectoplasm. On bursting, the cytoplasm flows away leaving the neuromotor apparatus intact (Pl. 42, Figs. 3 and 4). This consists of the axostyle with its capitulum continued to form the snout, the nucleus, parabasal body and the four flagella.

In fixed material the shape of the organism is variable but in most cases the anterior and posterior ends appear blunter than in life, also the organism becomes more spherical than ovoid. The axostyle shows more structure; there is a central striated core (Pl. 42, Figs. 3 and 4) which stains pale mauve with Mallory's Triple stain and grey with Heidenhain's iron-alum. Surrounding the core is an almost colourless sheath (Pl. 42, Figs. 3 and 4) which continues anteriorly over one side of the nucleus as the capitulum. The granules mentioned earlier in the living material stain bright red with Mallory's Triple stain after Zenker's or Schaudinn's fixative, but dark grey with Heidenhain's iron-alum. These granules resemble those described by Kirby (1936) in *C. nova,* which, according to him, probably correspond to the chromatic shield of other species.

The blepharoplast (Pl. 42, Fig. 3, blp.) shows up clearly as a dark granule (red with Mallory's Triple) at the base of the flagella. In some cases there appear to be two granules close together. The parabasal body stains readily and the parabasal thread can be seen

clearly, continuing to the blepharoplast (Pl. 42, Fig. 3).

The nucleus (Pl. 42, Figs. 3 and 4) is ovoid, being 10·7μ x 7·6μ in the larger specimens. After fixation the chromatin tends to become massed in the centre, leaving a clear space between it and the nuclear membrane. There is a karyosome present which is placed excentrically and surrounded by a clear area or halo (Pl. 42, Fig. 3). It stains bright red with Mallory's Triple, but black with Heidenhain's.

Discussion.

The genus *Caduceia,* belonging to the family Devescovenidae, was established by Franca in 1918 (*C. theobromae*). This species was redescribed by Grassé (1937) and Kirby (1938). In this paper Kirby draws attention to the similarity of the genera *Devescovina* and *Caduceia,* pointing out that the structural differences are in degree rather than kind. Nevertheless, these marked differences justify generic distinction.

According to the diagnosis of the genus *Caduceia* (Kirby, 1938, p. 2), the flagellate described above is included in this genus. Like *C. theobromae,* it resembles *Pseudo-devescovina uniflagellata* in its movements, size and shape, length of flagella and structure of trailing flagellum (slender and relatively short in *Caduceia* but flattened ribbon-like and long in *Devescovina*). Also the parabasal body is similar to that described for the genus *Caduceia,* with the parabasal thread attached to the blepharoplast.

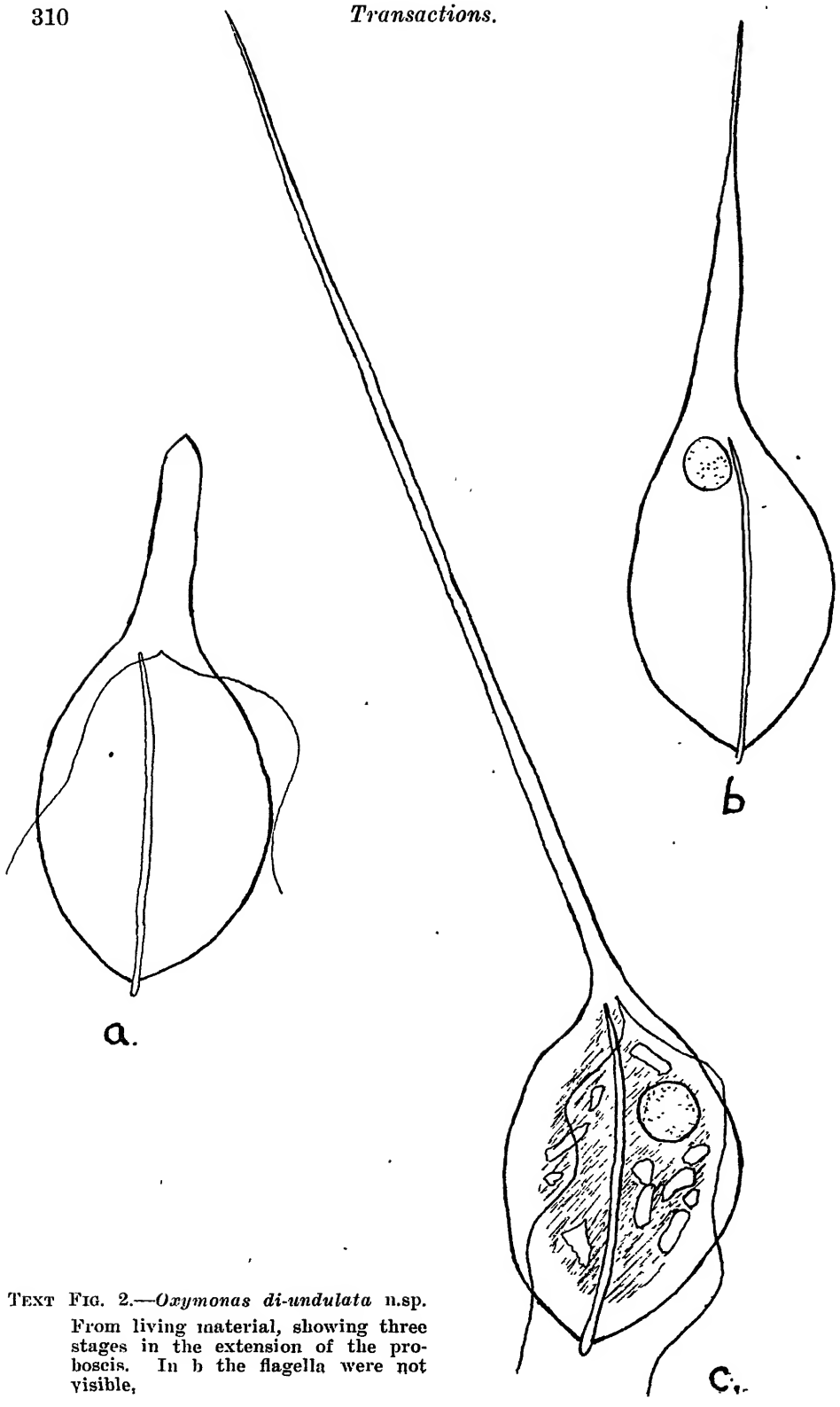
C. calotermidis resembles *C. nova* (Kirby, 1936) in the structure of the capitulum with the granules but differs in the tri-radiate structure of the anterior end of the axostyle, also in the absence of a cresta.

Oxymonas di-undulata, n.sp.

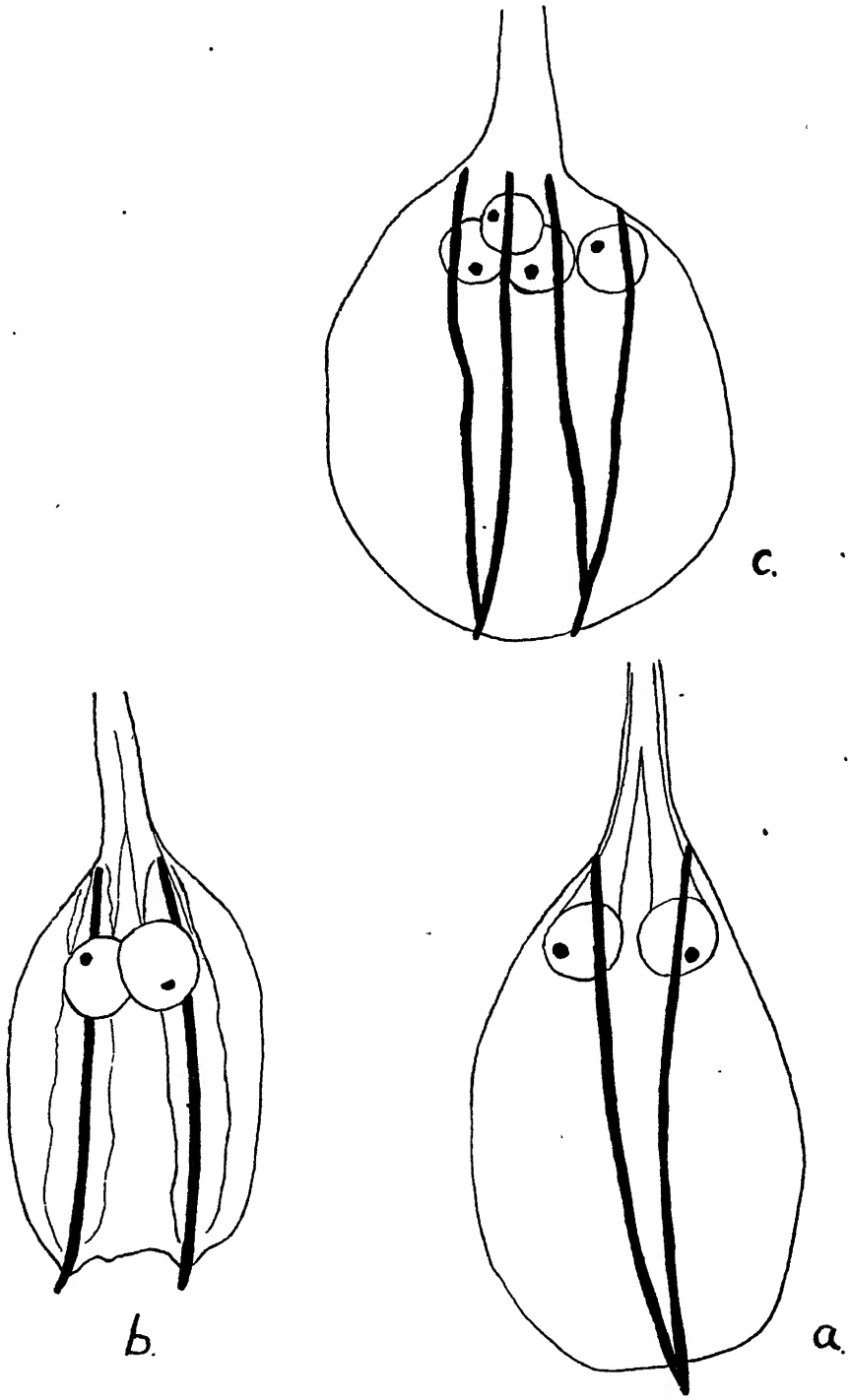
Host: Calotermes brouni.

Diagnosis: Length, 15·3μ–38·2μ; width, 12·2μ–27·5μ; body ovoid; nucleus anterior, spherical 5·6μ–6μ in diameter; two undulating membranes each with a flagellum arising from a single blepharoplast placed anteriorly; length of flagella variable, sometimes little longer than body, mostly shorter; axostyle which projects slightly from posterior end; extensile proboscis, when fully extended up to 180·5μ; proboscis when partly extended may have form of blunt cone; no proboscis sleeve visible.

The flagellate is ovoid, with an extensile proboscis which when extended is as much as nearly five times the length of the organism (Text Fig. 2c). In its retracted state this end, the anterior, is in the form of a blunt cone (Text Fig. 2a). The nucleus lies at the anterior end. There is a clear rod present which runs from the anterior end and projects slightly from the posterior. The cytoplasm is densely packed with wood particles. The size varies from 15·3μ to 38·2μ long and 12·2μ to 27·5μ wide, while the extended proboscis is up to 180·5μ long in large forms. There are two undulating membranes present, each bearing a flagellum. Each undulating membrane extends from the blepharoplast as much as three-quarters the length of the body (Pl. 43, Fig. 5). The pairs of flagella vary in length from one individual to another. The flagellates remained stationary but were seen to retract the proboscis. Some were observed to have adherent bacteria, but this was not common.



TEXT FIG. 2.—*Oxymonas di-undulata* n.sp. From living material, showing three stages in the extension of the proboscis. In b the flagella were not visible.



TEXT FIG. 3.—Some late dividing stages of *Oxymonus diundulata* n.sp. (a) Two nuclei present but axostyle not completely divided, still single at posterior end. (b) Two distinct axostyles present and four undulating membranes. (c) Division to form four nuclei and four axostyles has taken place without the formation of separate individuals. In (a) and (c) the undulating membranes were not visible.

In fixed material the clear rod (axostyle of Kofoid and Swezy) stains readily, bright red with Mallory's Triple after Zenker or Schaudinn's fixative and grey or black with Heidenhain's iron-alum, but when this is counter-stained with acid Fuchsin, it is again bright red. The proboscis is a clear structure and can be seen to continue up to the base of the nucleus. The axostyle lies over one side of the nucleus and is closely applied to the nuclear end of the proboscis. Just beyond the termination of the axostyle there is a darkly staining body, the blepharoplast, from which the flagella arise (Pl. 43, Fig. 6 blp.). The nucleus is spherical, varying in diameter from 5·6μ to 6μ. There is an eccentric karyosome with a distinct halo. Deeply staining large granules (Fig. 6g), the siderophile granules of Kofoid and Swezy, can be seen in the cytoplasm.

Some late dividing stages were observed (Text Fig. 3). One live specimen was seen to have two nuclei, two axostyles and four undulating membranes.

Discussion.

This flagellate has characters agreeing with those of the genus *Oxymonas,* but there are striking differences between it and the species which have been described elsewhere. Kofoid and Swezy (1926) consider the genus to have diverged from the condition seen in *Monocercomonas* (which is the tri-flagellate type) in two directions. The first is the duplication and modification of the neuromotor system. This duplication has not occurred in *O. di-undulata* as there is only one blepharoplast present, also, in this species there are but two flagella, each with its undulating membrane. It could be hardly, therefore, termed a triflagellate unless perhaps it has been reduced.

The second direction is in the development of a protrusible anterior process. The possession of this striking process also by *O. di-undulata* justifies the inclusion of this flagellate in the genus *Oxymonas.* No proboscis sleeve was observed.

Trichomonas agilis n.sp.

Host: Calotermes brouni.

Diagnosis: Length, 10μ–13·6μ; width, 7μ–9μ; pyriform to ovoid; three anterior flagella; fourth trailing flagellum three times length of body, with undulating membrane which does not extend to the posterior end; well marked costa; nucleus pyriform situated anteriorly.

The shape of the body varies from pyriform to ovoid in the more slender forms, the size being from 10μ to 13·6μ long and 7μ to 9μ wide. It tapers to a point anteriorly where there is situated a single blepharoplast from which arise three long flagella projecting anteriorly. The undulating membrane does not extend right to the posterior end (Pl. 44, Fig. 7). At the base of the undulating membrane is a well marked costa which stains bright red with Mallory's Triple stain (Kirby, 1931). The flagellum attached to the undulating membrane arises from the blepharoplast and continues free beyond the body about twice its length.

The movement of the organism is rapid and jerking. The axostyle is seen projecting from the posterior end (Pl. 44, Fig. 7), but it is not visible further within the body. The nucleus is situated close to the anterior end. It is pyriform, tapering anteriorly, and

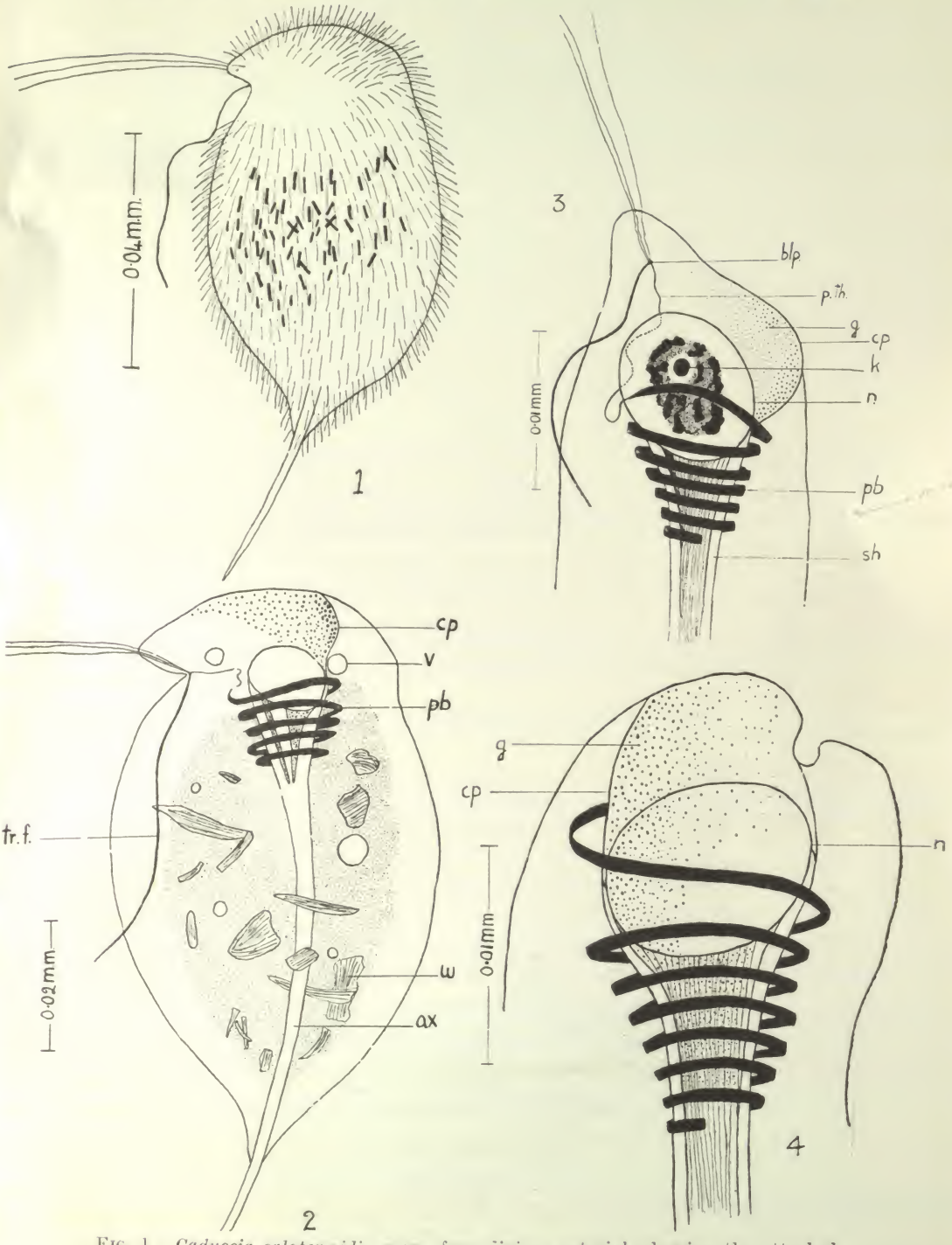


FIG. 1.—*Caduceia calotermidis*, n.sp., from living material, showing the attached spirochaetes and the rod-like micro-organisms.

FIG. 2.—*C. calotermidis*, n.sp., entire individual (spirochaetes omitted) from living specimen, stained with trace of alcoholic iodine.

FIG. 3.—Detail of neuromotor system. Mallory's Triple stain after Schaudinn. Granules, blepharoplast flagella and karyosome red. Parabasal body dark blue. Dotted line shows continuation of parabasal thread behind nucleus. Side view.

FIG. 4.—View from side opposite blepharoplast showing continuation of axostyle over one side of nucleus forming capitulum.

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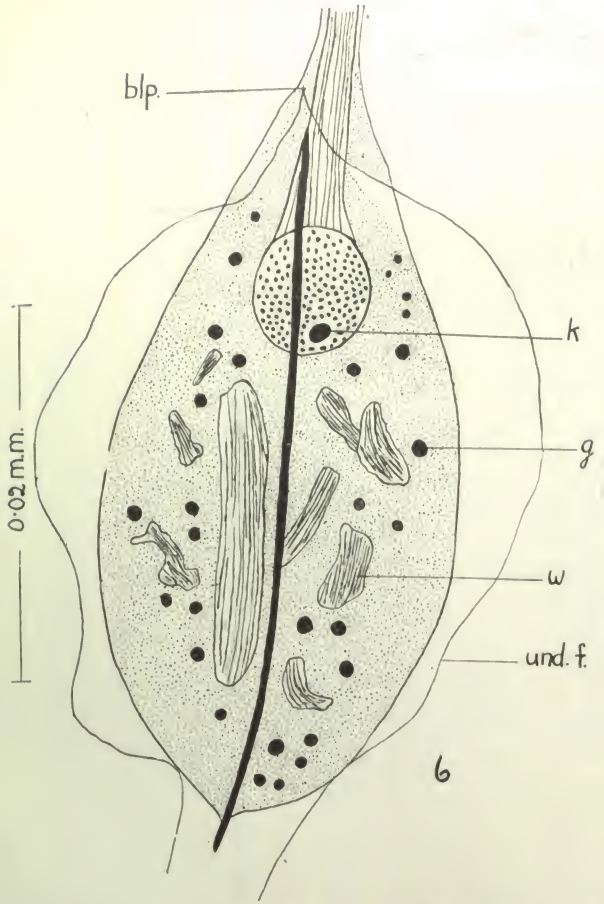

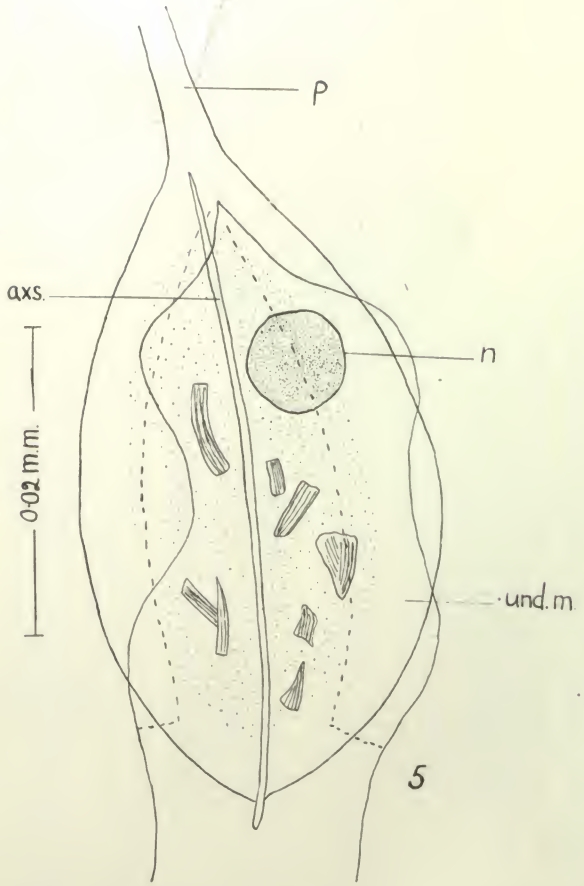

Fig. 5.—From living material. Length 30.6μ, width 22.9μ, nucleus 6μ in diameter, proboscis 53.5μ. Dotted lines indicate region of undulating membrane.

Fig. 6.—Entire individual, differently viewed from Fig. 5. (Proboscis not all included.) From specimen stained with Mallory's Triple after Zenker.

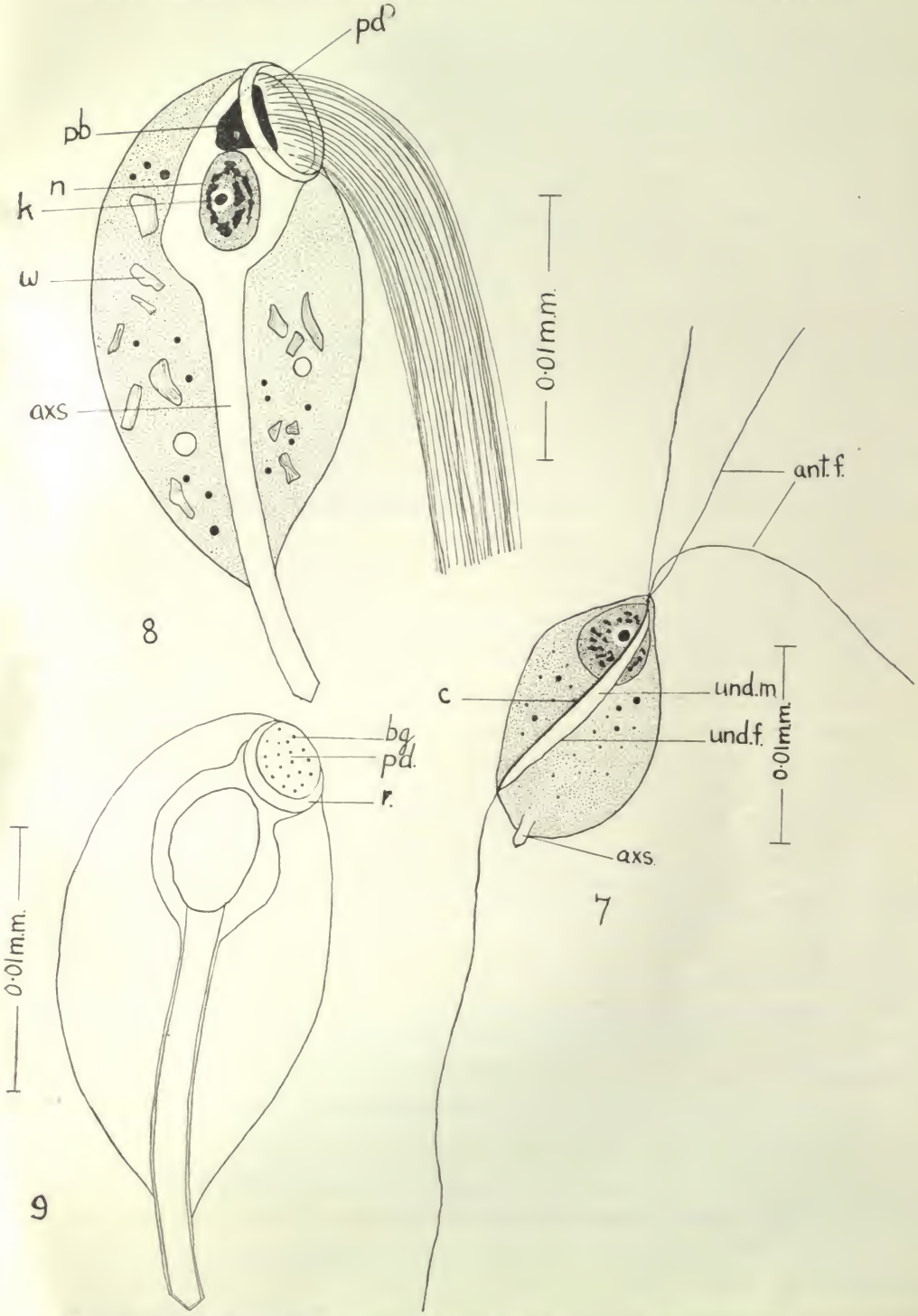

FIG. 7.—*Trichomonas agilis*, n.sp. (Mallory's Triple stain after Schaudinn.)

FIG. 8.—*Cyclojoenia australis*, n.gen., n.sp. (Mallory's Triple stain after Zenker's.)

FIG. 9.—*C. australis*. View looking into circular depression showing arrangement of basal granules. Flagella not shown.

measures one-third the length of the body. There is an eccentric karyosome with a halo. Numerous staining granules are present in the cytoplasm, but wood particles have not been identified.

Genus CYCLOJOENIA nov.

Joeniidae characterised by the possession of a circular plate-like depression bordered by a stout rim, from which arises the tuft of flagella. Flagella arise from basal granules evenly distributed over circular depression.

Genotype: *Cyclojoenia australis* n.sp.

Cyclojoenia australis n.sp.

Host: Stolotermes ruficeps.

Diagnosis: Length, 18·4μ–22·9μ; width, 10·7μ–15·3μ; pyriform, tapering posteriorly; about 20 flagella; stout axostyle which projects from posterior end; anteriorly it swells out and surrounds ovoid nucleus, it continues to rim surrounding circular depression; triangular parabasal body.

The flagellate is pyriform in shape, tapering posteriorly. The flagella project from a depression at the anterior end (Pl. 44, Figs. 8 and 9), which is bordered by a stout circular rim. There are at least 20 flagella, and these project backwards, lying alongside the body when the organism is at rest. They are slightly longer than the body. The organism moves rapidly in a jerking fashion by means of the flagella. They are raised towards the anterior end, the movement of each flagellum beginning proximally. They are then flicked rapidly to its side in metachronal succession.

The size ranges from 18·4μ to 22·9μ in length and from 10·7μ to 15·3μ in width. The axostyle (Pl. 44, Figs. 8 and 9), which is stout and sharply pointed, projects from the posterior end a distance of 6·1μ to 9μ in the large forms. The organism is xylophagous.

In the fixed and stained material the stout axostyle is almost colourless (pale mauve in Mallory's Triple stain). Anteriorly it swells out to surround the ovoid nucleus (Pl. 44, Figs. 8 and 9) and then appears to continue on to the circular rim surrounding the depression. The flagella appear to arise from the basal granules (Pl. 44, Fig. 9) evenly distributed over the circular plate-like depression. The karyosome present in the nucleus is eccentric and surrounded by a halo (Pl. 44, Fig. 8). There is a triangular parabasal body which lies above the nucleus (Pl. 44, Fig. 9 pb.) and the base of the triangle appears to be attached to the depressed area from which the flagella arise.

Discussion.

Cyclojoenia is very closely allied to the genus *Microjoenia* family Joeniidae, first described by Grassi on *M. hexamitoides*. He compares the anterior end to " an operculum of a lake containing fluid," from which the flagella arise in longitudinal rows. Cutler (1920) in his description of *M. axostylis* describes the flagella as arising from a series of granules arranged in two or three longitudinal rows. Both these species have a stout hyaline axostyle. *Cyclojoenia* resembles *Microjoenia* in size, in the possession of a stout hyaline axostyle which continues to the anterior end, and also in the possession of a tuft of flagella which arise from the anterior end. The basal granules

of the flagella, however, are arranged evenly on a depressed circular plate around which is a stout rim. This arrangement of the neuromotor system is considered sufficient to justify the establishment of a new genus, *Cyclojoenia*. Sutherland (1933) records two genera of the family *Joeniidae* from Australia; *Joenina pulchella* Grassi from *Porotermes grandis* Holmg. and *Staurojoenina assimilis* from *Calotermes oldfieldi* var. *chryseus*.

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ABBREVIATIONS.

(Figures based on free-hand sketches.)

ant.f.—anterior flagella.
ax.—axostyle.
bg.—basal granule.
blp.—blepharoplast.
c.—costa.
cp.—capitulum.
g.—granules.
k.—karyosome.
n.—nucleus.
p.—proboscis.
pb.—parabasal body.
pd.—plate-like depression.
pth.—parabasal thread.
r.—circular rim.
sh.—axostyle sheath.
trf.—trailing flagellum.
und.f.—undulating flagellum.
und.m.—undulating membrane.
v.—vacuole.
w.—wood.